Review

THE EMBO JOURNAL

iPS cells: a game changer for future medicine

Haruhisa Inoue[1,2,**], Naoki Nagata[1], Hiromi Kurokawa[1] & Shinya Yamanaka[1,3,*]

Abstract

The induced pluripotent stem cell (iPSC) technology is instrumental in advancing the fields of disease modeling and cell transplantation. We herein discuss the various issues regarding disease modeling and cell transplantation presented in previous reports, and also describe new iPSC-based medicine including iPSC clinical trials. In such trials, iPSCs from patients can be used to predict drug responders/non-responders by analyzing the efficacy of the drug on iPSC-derived cells. They could also be used to stratify patients after actual clinical trials, including those with sporadic diseases, based on the drug responsiveness of each patient in the clinical trials. iPSC-derived cells can be used for the identification of response markers, leading to increased success rates in such trials. Since iPSCs can be used in micromedicine for drug discovery, and in macromedicine for actual clinical trials, their use would tightly connect both micro- and macromedicine. The use of iPSCs in disease modeling, cell transplantation, and clinical trials could therefore lead to significant changes in the future of medicine.

Keywords cell transplantation; cohort study; disease modeling; future medicine; iPSC clinical trial; patient stratification
DOI 10.1002/embj.201387098 | Received 8 October 2013 | Revised 27 November 2013 | Accepted 6 December 2013 | Published online 5 February 2014
EMBO Journal (2014) 33, 409–417

See the Glossary for abbreviations used in this article.

Introduction

Like any other scientific advance, the iPSC technology (Fig 1) was established on the basis of numerous findings by past and current scientists in related fields (Yamanaka, 2012). Although the detailed mechanisms underlying the reprogramming process during iPSC generation are still being elucidated, the final products, which had previously been inaccessible, show promise for multiple purposes related to understanding disease mechanisms and strengthening the skills critical for patient treatment (Takahashi & Yamanaka, 2013). Although the iPSC technology still requires improvements and refinement, its contributions to disease modeling and cell transplantation studies are already well-recognized. New technologies, including direct cellular reprogramming and gene-editing, are optimizing the application of the iPSC technology for future medicine.

From this time onward, the progress in iPSCs and associated technologies is expected to engender novel criteria for patient stratification and for the regulation of clinical trials based on drug responsiveness, and the iPSC technology will contribute to more precise medicine in the future.

Disease modeling

The study of disease mechanisms and therapies is being enhanced by iPSC technology-based disease modeling. Following the first report of human iPSCs in 2007, the initiation of iPSC disease modeling was started by the generation of iPSCs using somatic cells from aged patients (Dimos *et al*, 2008) and patients with many types of diseases (Park *et al*, 2008), and the variety of diseases being modeled continues to grow (Supplementary Table S1). It is known that drugs used in animal models are not always effective for human beings (Inoue & Yamanaka, 2011). For example, a systematic study of inflammation showed that the gene expression changes in mice had little correlation with the changes seen in humans (Seok *et al*, 2013). Many genetic variants associated with human diseases are located in non-coding regions that show relatively little evolutionary conservation, which means that their introduction in animals is unlikely to result in phenotypes relevant to human diseases (Merkle & Eggan, 2013). Moreover, it may also be difficult to simultaneously recapitulate the gain and loss of function of the disease-causative proteins in human diseases (Winklhofer *et al*, 2008) by generating simple transgenic or knockout mice. In addition, one of the statin drugs, compactin, barely reached a human clinical trial level, since it was not effective for rats, in spite of being properly validated in humans (Tobert, 2003). Such discrepancies highlight the significance of using human cells for drug evaluation.

Of prime importance is the establishment of a *de facto* standard of disease modeling, including the quality control of iPSCs, as shown by previous reports summarized in Supplementary Table S1. However, iPSC disease modeling is faced with several obstacles. It has been revealed that heterogeneous cell populations exist after differentiation from iPSCs, and cells are not able to synchronize the developmental stages of cell populations (Kitaoka *et al*, 2011). These disparities in the differentiation efficiency and maturation

1 Center for iPS Cell Research and Application(CiRA), Kyoto University, Kyoto, Japan
2 JST CREST, Japan Science and Technology Agency, Saitama, Japan
3 Gladstone Institute of Cardiovascular Disease, San Francisco, CA, USA
*Corresponding author. Tel: (+81) 75 366 7044; Fax: (+81) 75 366 7042; E-mail: yamanaka@cira.kyoto-u.ac.jp
**Corresponding author. Tel: (+81) 75 366 7036; Fax: (+81) 75 366 7094; E-mail: haruhisa@cira.kyoto-u.ac.jp

Glossary

ALS	amyotrophic lateral sclerosis
Aβ	amyloid β
BiP	immunoglobulin heavy-chain binding protein
ESC	embryonic stem cell
iPSC	induced pluripotent stem cell
GSK-3β	glycogen synthase kinase-3β
HLA	human leukocyte antigen
p-tau	phospho-tau (Thr231)
PRDX4	peroxiredoxin-4
t-tau	total tau

among clones are considered to originate from incomplete reprogramming, genetic background variability (Soldner & Jaenisch, 2012), epigenetic memory (Kim *et al*, 2010) or erosion of X-chromosome inactivation (Mekhoubad *et al*, 2012). There are several points that need to be addressed to overcome these obstacles facing iPSC disease modeling (Table 1), as follows:

Robust differentiation or purification/enrichment of target cells

Using a cell-specific promoter or cell-surface antigen, it is possible to isolate and obtain target cells with the same degree of maturation (Kitaoka *et al*, 2011; Egawa *et al*, 2012; Sandoe & Eggan, 2013; Yu *et al*, 2013), even though perfect purity is not yet possible.

One of the robust differentiation methods is to induce transcription factors for direct differentiation, *i.e.* direct reprogramming, which can be used to induce specific types of cells, including neurons (Vierbuchen, *et al*, 2010; Son *et al*, 2011; Qiang *et al*, 2013), cardiomyocytes (Ieda *et al*, 2010), blood cell progenitors (Szabo *et al*, 2010), hepatocyte-like cells (Huang *et al*, 2011; Sekiya & Suzuki, 2011) and cartilaginous tissue (Hiramatsu *et al*, 2011), as well as to determine the germ cell fate (Nakaki *et al*, 2013). Using this approach, disease modeling is possible (Qiang *et al*, 2011; Son *et al*, 2011; Rhinn *et al*, 2013). The major advantage of the direct cellular reprogramming/induced cell technology is that it works well in large cohorts of samples. On the other hand, there is a limit in the number of original somatic cells used as a resource, meaning that, while the induced cells are suitable for a large cohort analysis, they are not indicated for use in a large-scale analysis using a single line.

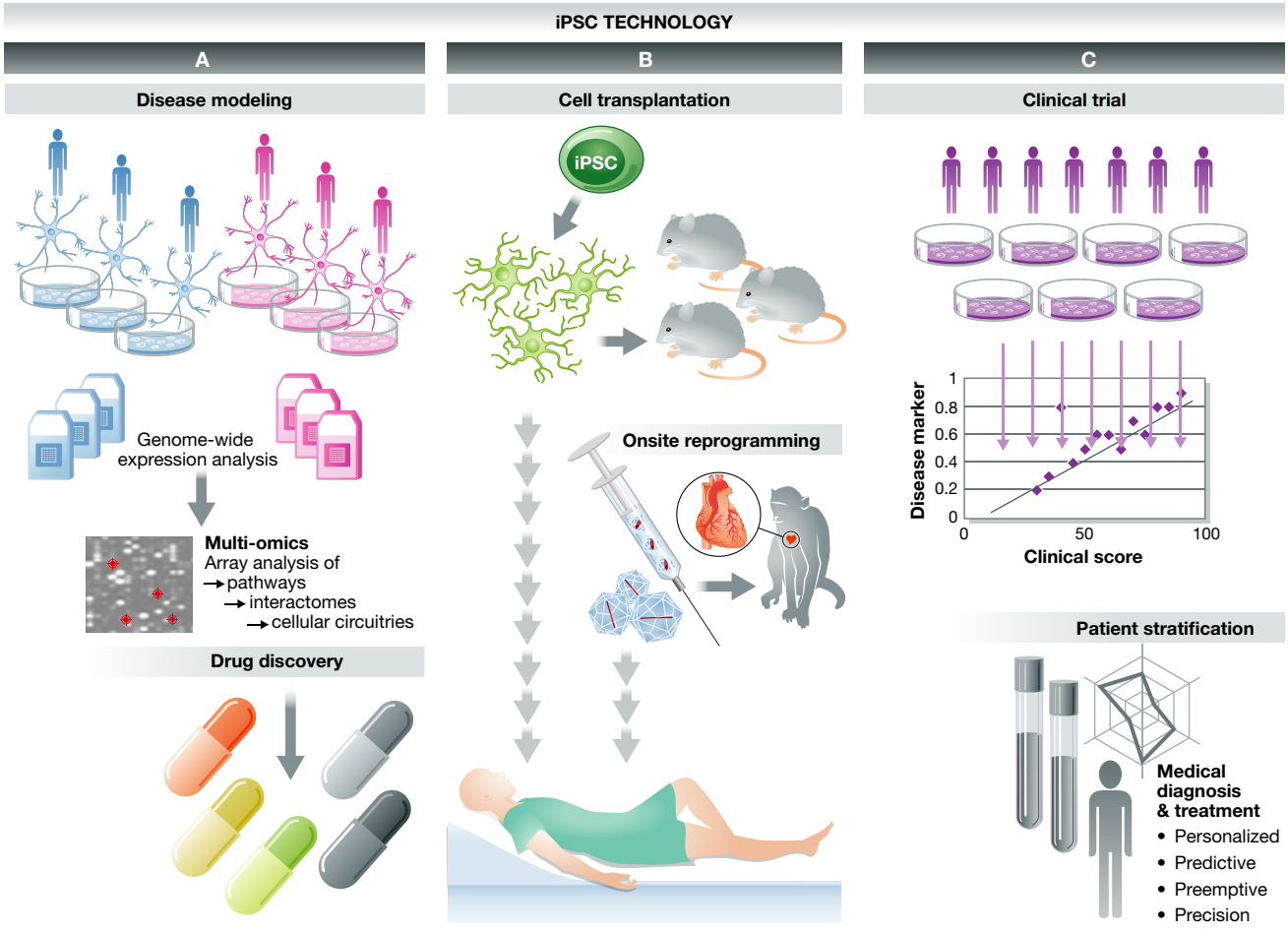

Figure 1. iPSC technology contributes to 'Disease modeling', 'Cell transplantation', and 'Clinical trial'.
(A) Disease modeling using patient iPSCs for 'Multi-omics' and 'Drug discovery'. (B) Instead of 'Cell transplantation', 'Onsite reprogramming' may be applied. (C) iPSCs from a large cohort of patients can be applied to 'Clinical trial' and 'Patient stratification'.

Table 1. Points in disease modeling

1. Robust differentiation or purification/enrichment of target cells
2. Mimicking of disease niche by additional conditions
3. A highly sensitive detection system
4. Optimal control setting
5. Validation with human sample and/or other disease models

The direct cellular reprogramming/induced cell technology also has advantages in terms of the multi-sample analysis, cost and time, and cellular maturation; iPSCs are preferable in terms of gene-editing, the fact that they are an unlimited resource, and because they can differentiate into a great variety of cells. Although direct cellular programming was revealed to have the disadvantage of not being able to generate a renewable source of programmed cells, several labs have recently shown that programming can be achieved for a proliferating population of neural precursor cells that can then be propagated and subsequently differentiated into mature neurons and glia (Marchetto & Gage, 2012). In addition, the fusion of the direct cellular reprogramming technology with iPSCs would produce a hybrid technology that promotes the merits of both technologies (Imamura & Inoue, 2012), and this has already been reported for neurons (Hester *et al*, 2011; Zhang *et al*, 2013), hepatoblasts (Inamura *et al*, 2011) and myocytes (Tanaka *et al*, 2013). This hybrid technology will be even more useful after iPSCs can be generated more rapidly, easily and inexpensively.

Mimicking of disease niches by additional conditions

Genetic factors may not manifest functional defects in iPSC models under basal culture conditions, and might require the use of stressors to challenge the cell cultures (Kim *et al*, 2013). In addition, many neurodegenerative diseases are late-onset diseases, and their key phenotypes may not manifest themselves easily within a short period of time in culture. To mimic the aging process, cellular stress can be imposed, or trophic factors can be depleted.

Selective susceptibility of neuronal cell types in many neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), can be induced by pathological changes in the neurons, as well as in their interacting partners (Xu & Zhong, 2013).

A highly sensitive detection system

The challenge of elucidating subtle but significant phenotypes in long-term cultures requires the application of multiple complementary readouts. A real-time single-cell longitudinal survival analysis using fluorescent reporter genes has enabled the determination of differences in cellular survival (Bilican *et al*, 2012). Single-cell expression profiling should clarify the levels of population heterogeneity within *in vitro* cultures, and advances in media culture platforms and automated cell processing should provide the accuracy and consistency that will be required (Citri *et al*, 2012; Yu *et al*, 2013). In addition, specific antibodies against intracellular pathogens have been tested (Kondo *et al*, 2013), and will continue to be developed.

Optimal control settings

Recent genome-wide association studies have demonstrated that every person possesses disease-relevant single-nucleotide polymorphisms, and it is therefore impossible to categorically define iPSCs that represent perfect non-disease control. Nonetheless, we think that deductive and inductive control (Inoue & Yamanaka, 2011) are valid for deriving iPSC-positive (disease) and negative (non-disease) controls. Deductive controls would include non-disease iPSC/embryonic stem cell (ESC) lines, together with gene-edited, isogenic iPSC lines (see Supplementary Table S1). Many methods have been used in the past for gene targeting in pluripotent stem cell lines (Sandoe & Eggan, 2013). Using the isogenic control, a labor reduction and noise cancellation from clonal variations would be possible. On the other hand, multiple clones must be analyzed so as to avoid off-target effects even in the isogenic control lines. If isogenic cell lines are compared, there is no clear answer at present regarding how many isogenic pairs should be analyzed (Merkle & Eggan, 2013).

In addition, when deductive clones are generated by introducing mutations into control human iPSC/ESC lines, protective alleles may intercept the expression of disease-phenotypes.

Validation with human samples and/or other disease models

Although iPSC technology provides novel resources, there is still room for improvement. It is still necessary to better validate the phenotypes with other systems, and to confirm that the phenotypes do not stem from the fragility of the technology by using human samples and other models. In this regard, there are some experimental conditions that only the iPSC technology can provide, such as the co-culture of disease cells and healthy control cells.

Cell transplantation

The iPSC technology is contributing to the study of cell transplantation. The advantages of iPSC are as follows: Autologous cells, which suppress the risks of rejection and infection, could be used; diseases caused by single gene defects could be addressed by made-to-order gene replacement in cells and allogenic cells from healthy people could be used.

A report of a mouse model of sickle-cell anemia, a genetic blood disorder caused by a defect in the β-globin gene, provided a proof-of-concept illustration of the therapeutic use of iPSCs (Hanna *et al*, 2007). In that study, a mutant iPSC line with gene correction by homologous recombination was used for transplantation into mutant mice to cure the disease. This exemplified the potential of regenerative medicine using iPSCs (Takahashi & Yamanaka, 2013). It was shown using a non-human primate PD model that autografts caused only a minimal immune response in the primate brain, and autografts have an advantage over allografts even at immunologically privileged sites (Morizane *et al*, 2013).

In contrast, the use of autologous iPSCs from every individual would necessarily result in high medical costs. Since it takes more than three months to generate iPSCs using the current methods, such a time line is hardly optimal for the effective treatment of certain disorders, such as spinal cord injury (Nakamura & Okano, 2013; Takahashi & Yamanaka, 2013). Furthermore, autografts from sporadic disease cases might harbor disease phenotypes. For these reasons, the importance of considering the use of allogeneic iPSC lines for transplantation therapy must be emphasized. Multiple iPSC clones could easily be generated from the diversity of donor candidates with validated health conditions and the types of human leukocyte antigen (HLA) needed for generating clinical-grade iPSC

clones (Takahashi & Yamanaka, 2013). Matching the three major types of HLA loci between the recipient and donor is expected to result in less immune rejection after transplantation following bone marrow transplantation. One of the most feasible methods for iPSC therapy, therefore, will be based on the collection of iPSC stocks derived from various HLA-homozygous donors under Good Manufacturing Practice (GMP) compliance (Nakajima *et al*, 2007; Okita *et al*, 2011; Takahashi & Yamanaka, 2013).

Some new technologies related to cell transplantation have been emerging. Dynamic patterning and structural self-formation of complex organ buds in 3D stem cell culture, including the generation of various neuroectodermal and endodermal tissues, have been discovered (Sasai, 2013). Another example of tissue generation was illustrated by the injection of wild-type rat pluripotent stem cells into the blastocysts of Pdx1-deficient mice, which are unable to grow a pancreas, and this resulted in the generation of normally functioning rat pancreatic tissue (Kobayashi *et al*, 2010). The self-organization of tissue development, a major advantage of pluripotency-mediated strategies, would be valuable not only for the next generation of organ transplantation, but also for disease modeling (Takahashi & Yamanaka, 2013). Furthermore, on-site reprogramming technology (Yu *et al*, 2013) has already been applied to the production of β-cells (Zhou *et al*, 2008) and cardiomyocytes (Qian *et al*, 2012; Song *et al*, 2012; Inagawa *et al*, 2012), and it will progress following the development of improved delivery methods. Immunological cells, including T cells (Nishimura *et al*, 2013; Vizcardo *et al*, 2013; Wakao *et al*, 2013), are also expected to be used for cell therapy.

New iPSC-based medicine

The iPSC technology has opened new possibilities for generating continuous supplies of progenitor cells for toxicity screening. A toxicity assay using iPSCs would be the first step in clinical trials (iPSC clinical trials). Proof-of-concept toxicity studies performed with human iPSC-derived differentiated cell types (Guo *et al*, 2011; Medine *et al*, 2013) support the concept of large-scale human cell-based toxicity screens. Drug-induced side effects in the liver, heart and brain have been thoroughly studied. It is both feasible and effective to use iPSC-derived cells between the drug discovery phase and development phase as clinical trial 'Phase 0.5'. However, there are several limitations to the sourcing of these cells, such as the achievement of fully mature phenotypes.

While stem cell-based hepatocyte toxicity assays are still at an early stage of development, proof-of-concept studies of known toxicants have been performed (Scott *et al*, 2013). It was also demonstrated that iPSC-derived cardiomyocytes could be treated using a subset of known arrhythmogenic drugs (Guo *et al*, 2011; Lahti *et al*, 2012). Applying electrophysiology methods to study the response of iPSC-derived cardiomyocytes to drug treatment provided prospective results, but such results are limited to the different experimental setups and the number of drugs evaluated in each study has been small (Deshmukh *et al*, 2012).

The results of these preliminary studies indicate that the toxic compounds that are already well known and have known mechanisms of action should be tested first with iPSC-derived cells, and the requirements, properties and differentiation protocols for the cells derived from standard iPSCs should be decided based on these findings.

In contrast to the drug-induced hepatotoxicity and cardiotoxicity, the mechanisms of which are relatively easy to discern, the reverse-translation of neuronal side effects into discrete cellular mechanisms and toxicity pathways for *in vitro* screening remains a challenge. However, proof-of-concept studies using the high-content analysis of different cell types are expected to be conducted by analyzing the features of neurodevelopment, including neurite outgrowth and synaptogenesis (Scott *et al*, 2013).

In an aging society, one of the unmet medical needs is that of drug development for Alzheimer's disease (AD). We previously analyzed the neural cells from AD patient iPSCs, and found that there are subgroups among AD cells. This indicates that clinical AD may need to be reclassified into different sub-types, and that the prediction of the drug responsiveness may be possible based on the different sub-types (Kondo *et al*, 2013). If we scale up the study, such as by performing an iPSC clinical trial of Phase 1.5, and generate AD and control iPSCs from a larger cohort of patients, it may become possible to select responders and non-responders to specific drugs, leading to a Phase II clinical trial only for responders. Or we could identify a responder marker, after actual clinical trials of a drug, using the iPSCs from responders and non-responders in the trials. This identified marker could then be used to enrich the responders in the next step, leading to higher success rates. Another report also showed that the neurons generated from iPSCs derived from four AD patients showed significantly higher levels of Aβ40 in the culture medium of the neurons generated from three of the four patients, supporting the concept of the heterogeneity of AD (Israel *et al*, 2012).

There have also been other reports showing patient stratification with the differential drug responsiveness (Table 2). For example, several clinical trials for spinal muscular atrophy (SMA) have been conducted. The completed clinical trials demonstrated that valproic acid (VPA) is only beneficial to a restricted subset of SMA patients, and that there are responders and non-responders (Garbes *et al*,

Table 2. Responder selection in a disease with differential drug responsiveness

Disease	Drug	Marker	Total (*n*) control/disease/responder	Reference
Retinitis pigmentosa	α-Tocopherol	RP9 mutation	6 1/5/2	Jin *et al* (2011)
Alzheimer's disease	β-secretase inhibitor γ-secretase inhibitor	Aβ(1-40), GSK-3β, p-tau/t-tau	6 2/4/3	Israel *et al* (2012)
Spinal muscular atrophy	VPA	CD36	2 0/2/1	Garbes *et al* (2013)
Alzheimer's disease	DHA	Aβ oligomer, BiP, PRDX4	7 3/4/2	Kondo *et al* (2013)

2013). The drug responsiveness of neuronal cells derived from responder iPSCs and non-responder iPSCs to VPA was compatible with the results of the clinical trials (Garbes *et al*, 2013). Although large clinical trials have been conducted with α-tocopherol (vitamin E), no statistically significant change in visual function of retinitis pigmentosa (RP) patients was found (Jin *et al*, 2011). The underlying mutations causing the disease in the patients tested in the above clinical trials were not revealed, and the variability of individual responses to these drugs is unknown. However, a recent study showed that the rod cells derived from iPSCs of RP patients showed differential responsiveness to vitamin E, suggesting that RP may be divided into subgroups by the drug responsiveness (Jin *et al*, 2011). Therefore, the iPSC technology can contribute to micromedicine, including drug discovery based on cellular and molecular analyses, as well as to macromedicine, including patient stratification based on cellular and molecular analyses of participants in clinical trials or cohort studies (Fig 2).

iPSC clinical trials may make it possible to identify a drug-responsive subgroup of patients with a specific disease, and a more precise Phase II clinical trial could thus be performed (Fig 3). The iPSC clinical trial approach could be applied to a large cohort analysis with medical records and genome information. A genome analysis provides ample information, but it is hard to establish sporadic disease models on the basis of such findings. We found that the Aβ metabolisms differed according to the respective APP mutations (Kondo *et al*, 2013), and an APP mutation that protects against Alzheimer's disease was recently reported (Jonsson *et al*, 2012). These findings suggest that, besides the genomic analyses, iPSC-derived cells would be useful for precise analysis of the individual genes and proteins. In addition when a new mutation is found, an analysis of target cells derived from iPSCs would provide an answer to the question of whether the mutation is pathogenic or not (Egashira *et al*, 2013).

We believe that iPSCs can be game changer that will help to avoid the possibility that a candidate drug tested in a clinical trial might be irrationally dropped based on the old rules. The previous clinical diagnoses are now changing based on the results of the genome analysis and multi-omics analysis of patient samples, including iPSCs. In addition, patients can be stratified based on the drug responsiveness of their iPSC-derived cells, which, as a consequence, could lead to a new type of diagnosis and stratification. A genetic diagnosis of sporadic diseases is difficult, but a drug response-based diagnosis might be possible based on the effectiveness of drugs in clinical trials (Fig 4). The required conditions for iPSCs used *in vitro* are different from those used for cell transplantation. The development of technologies for generating budget-conscious personalized iPSCs rapidly, homogenously and easily will be required for such iPSC-based clinical trials. To make iPSC clinical trials a reality, the regulatory system would need to be changed.

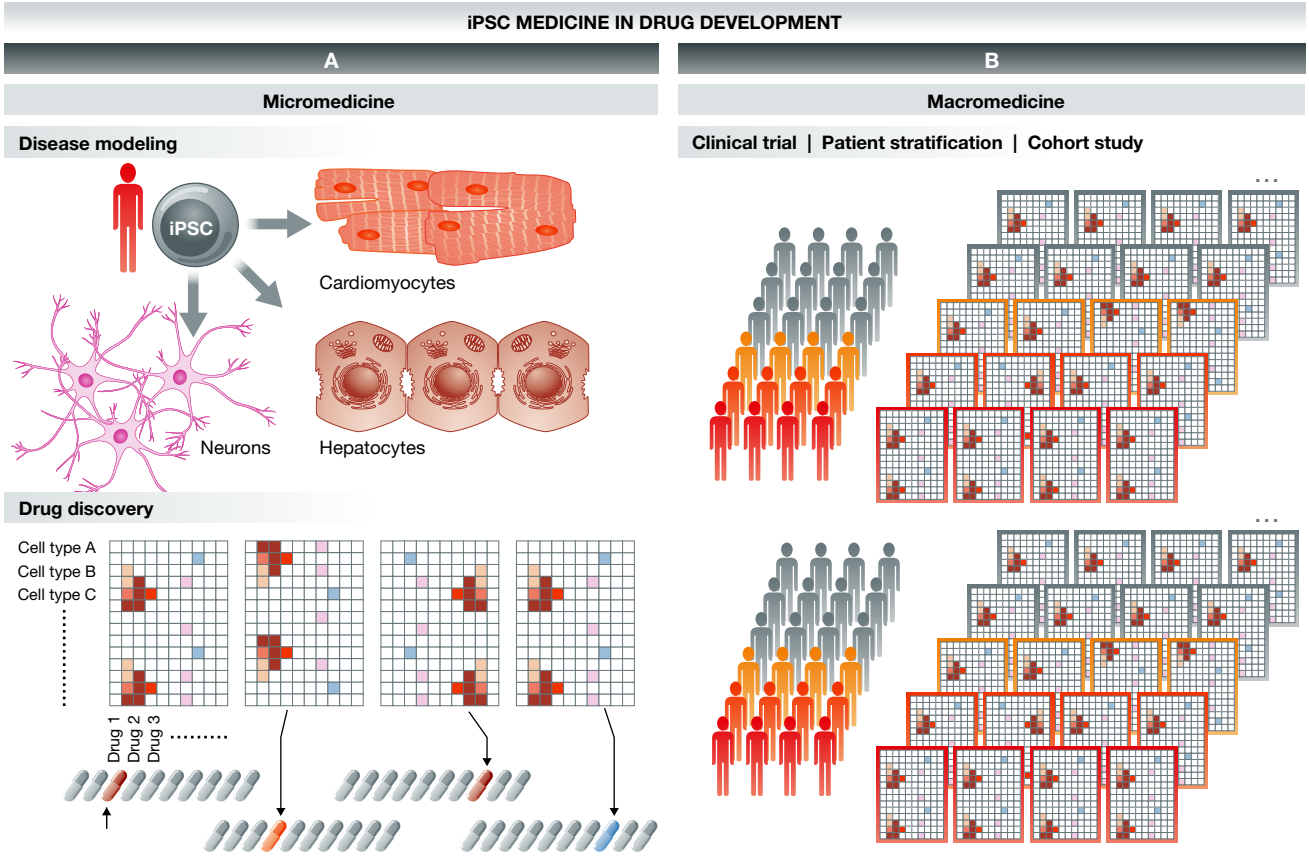

Figure 2. iPSC medicine in drug development ranges from 'Micromedicine' to 'Macromedicine'.

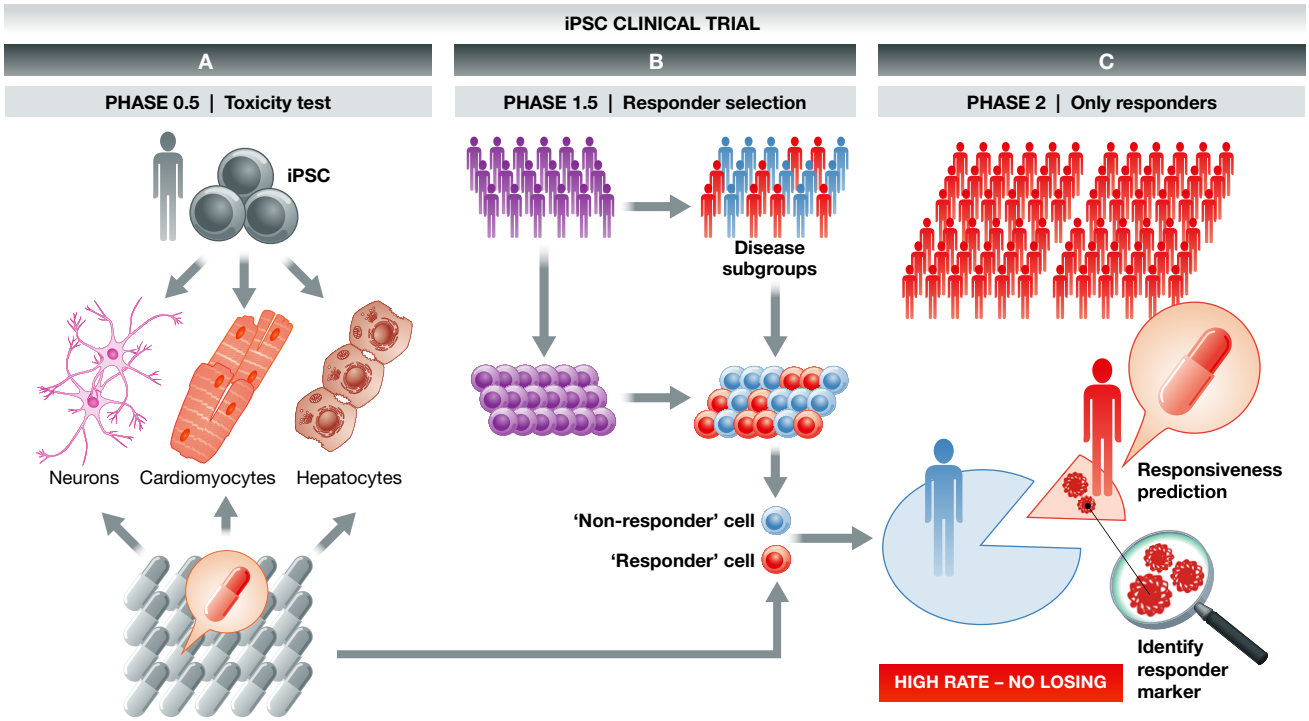

Figure 3. 'iPSC clinical trial' for phase 0.5, 1.5, and 2.

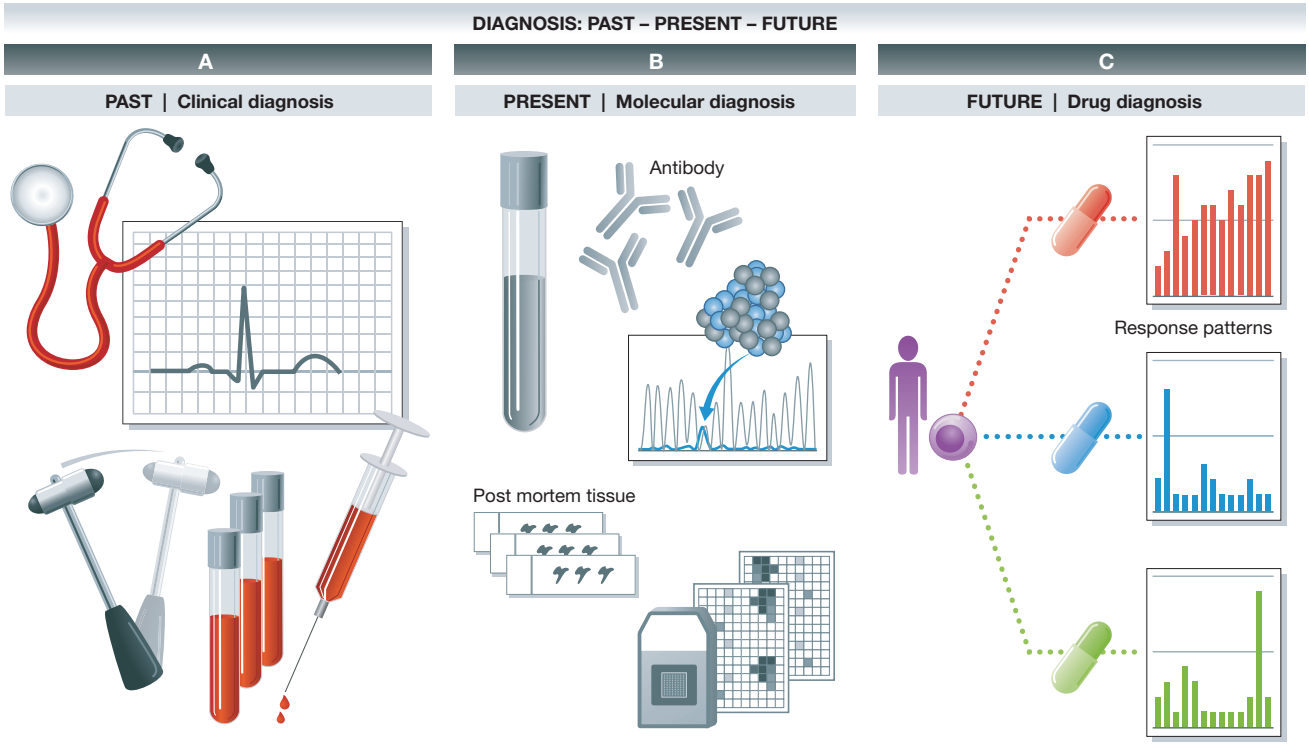

Figure 4. Patient diagnoses in the past, present, and future.

Conclusions

According to the current medical technologies, after the onset of a disease, patients are diagnosed and treated. Although the significance of the prevention of chronic diseases is well recognized, preventive medicine, which has been developed based on epidemiological studies and statistics, cannot be applied to individuals, and cannot provide a precise diagnosis or individualized therapeutics. Theoretically, everybody has disease-relevant SNPs, and every person has an increased change of becomes a patient during his/her lifetime. The iPSC technology will contribute to personalized, predictive, preemptive (Zerhouni, 2005; Auffray *et al*, 2009) and precision medicine (Mirnezami *et al*, 2012).

Supplementary information for this article is available online: http://emboj.embopress.org

Acknowledgements

We would like to express our sincere gratitude to all of our coworkers and collaborators, to Takayuki Kondo for drawing the figures and for critical discussions, to Kazuya Goto for editing Supplementary Table S1, and to Yoko Miyake, Rie Kato, Saki Okamoto, Eri Minamitani, Sayaka Takeshima, Ryoko Fujiwara, Katsura Noda, and Kazumi Murai for their valuable administrative support. We apologize for any data that we were unable to cite because of space limitations. This research was funded in part by a grant from the Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program) of the Japan Society for the Promotion of Science (JSPS) to S.Y., from Research Center Network for Realization of Regenerative Medicine of the Japan Science and Technology Agency (JST) to S.Y. and H.I. from CREST to H.I., from Research on Applying Health Technology, the Ministry of Health, Labour and Welfare of Japan to H.I., from a Grant-in-Aid for Scientific Research on Innovative Area Foundation of Synapse and Neurocircuit Pathology (22110007) from the Ministry of Education, Culture, Sports, Science and Technology of Japan to H.I., from the Japan Research Foundation for Clinical Pharmacology to H.I, from The Mochida Memorial Foundation for Medical and Pharmaceutical Research to H.I., and from Intramural Research Grant (24-9) for Neurological and Psychiatry Disorders of NCNP to H.I.

Author contributions

SY: conceived this project. HI: designed the figures and the concepts. SY and HI: wrote the paper. NN and HK: collected and analyzed the data of Supplementary Table S1.

Conflict of interest

S.Y. is a member without salary of the scientific advisory boards of iPierian, iPS Academia Japan, Megakaryon Corporation and HEALIOS K. K. Japan.

References

Auffray C, Chen Z, Hood L (2009) Systems medicine: the future of medical genomics and healthcare. *Genome Med* 1: 2

Bilican B, Serio A, Barmada SJ, Nishimura AL, Sullivan GJ, Carrasco M, Phatnani HP, Puddifoot CA, Story D, Fletcher J, Park IH, Friedman BA, Daley GQ, Wyllie DJ, Hardingham GE, Wilmut I, Finkbeiner S, Maniatis T, Shaw CE, Chandran S (2012) Mutant induced pluripotent stem cell lines recapitulate aspects of TDP-43 proteinopathies and reveal cell-specific vulnerability. *Proc Natl Acad Sci USA* 109: 5803 – 5808

Citri A, Pang ZP, Südhof TC, Wernig M, Malenka RC (2012) Comprehensive qPCR profiling of gene expression in single neuronal cells. *Nat Protoc* 7: 118 – 127

Deshmukh RS, Kovács KA, Dinnyés A (2012) Drug discovery models and toxicity testing using embryonic and induced pluripotent stem-cell-derived cardiac and neuronal cells. *Stem Cells Int* 2012: 379569

Dimos JT, Rodolfa KT, Niakan KK, Weisenthal LM, Mitsumoto H, Chung W, Croft GF, Saphier G, Leibel R, Goland R, Wichterle H, Henderson CE, Eggan K (2008) Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science* 321: 1218 – 1221

Egashira T, Yuasa S, Fukuda K (2013) Novel insights into disease modeling using induced pluripotent stem cells. *Biol Pharm Bull* 36: 182 – 188

Egawa N, Kitaoka S, Tsukita K, Naitoh M, Takahashi K, Yamamoto T, Adachi F, Kondo T, Okita K, Asaka I, Aoi T, Watanabe A, Yamada Y, Morizane A, Takahashi J, Ayaki T, Ito H, Yoshikawa K, Yamawaki S, Suzuki S *et al* (2012) Drug screening for ALS using patient-specific induced pluripotent stem cells. *Sci Transl Med* 4: 145ra104 .

Garbes L, Heesen L, Hölker I, Bauer T, Schreml J, Zimmermann K, Thoenes M, Walter M, Dimos J, Peitz M, Brüstle O, Heller R, Wirth B (2013) VPA response in SMA is suppressed by the fatty acid translocase CD36. *Hum Mol Genet* 22: 398 – 407

Guo L, Abrams RM, Babiarz JE, Cohen JD, Kameoka S, Sanders MJ, Chiao E, Kolaja KL (2011) Estimating the risk of drug-induced proarrhythmia using human induced pluripotent stem cell-derived cardiomyocytes. *Toxicol Sci* 123: 281 – 289

Hanna J, Wernig M, Markoulaki S, Sun CW, Meissner A, Cassady JP, Beard C, Brambrink T, Wu LC, Townes TM, Jaenisch R (2007) Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science* 318: 1920 – 1923

Hester ME, Murtha MJ, Song S, Rao M, Miranda CJ, Meyer K, Tian J, Boulting G, Schaffer DV, Zhu MX, Pfaff SL, Gage FH, Kaspar BK (2011) Rapid and efficient generation of functional motor neurons from human pluripotent stem cells using gene delivered transcription factor codes. *Mol Ther* 19: 1905 – 1912

Hiramatsu K, Sasagawa S, Outani H, Nakagawa K, Yoshikawa H, Tsumaki N (2011) Generation of hyaline cartilaginous tissue from mouse adult dermal fibroblast culture by defined factors. *J Clin Invest* 121: 640 – 657

Huang P, He Z, Ji S, Sun H, Xiang D, Liu C, Hu Y, Wang X, Hui L (2011) Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors. *Nature* 475: 386 – 389

Ieda M, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, Srivastava D (2010) Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell* 142: 375 – 386

Imamura K, Inoue H (2012) Research on neurodegenerative diseases using induced pluripotent stem cells. *Psychogeriatrics* 12: 115 – 119

Inagawa K, Miyamoto K, Yamakawa H, Muraoka N, Sadahiro T, Umei T, Wada R, Katsumata Y, Kaneda R, Nakade K, Kurihara C, Obata Y, Miyake K, Fukuda K, Ieda M (2012) Induction of cardiomyocyte-like cells in infarct hearts by gene transfer of Gata4, Mef2c, and Tbx5. *Circ Res* 111: 1147 – 1156

Inamura M, Kawabata K, Takayama K, Tashiro K, Sakurai F, Katayama K, Toyoda M, Akutsu H, Miyagawa Y, Okita H, Kiyokawa N, Umezawa A, Hayakawa T, Furue MK, Mizuguchi H (2011) Efficient generation of hepatoblasts from human ES cells and iPS cells by transient overexpression of homeobox gene HEX. *Mol Ther* 19: 400 – 407

Inoue H, Yamanaka S (2011) The use of induced pluripotent stem cells in drug development. *Clin Pharmacol Ther* 89: 655–661

Israel MA, Yuan SH, Bardy C, Reyna SM, Mu Y, Herrera C, Hefferan MP, Van Gorp S, Nazor KL, Boscolo FS, Carson CT, Laurent LC, Marsala M, Gage FH, Remes AM, Koo EH, Goldstein LS (2012) Probing sporadic and familial Alzheimers disease using induced pluripotent stem cells. *Nature* 482: 216–220

Jin ZB, Okamoto S, Osakada F, Homma K, Assawachananont J, Hirami Y, Iwata T, Takahashi M (2011) Modeling retinal degeneration using patient-specific induced pluripotent stem cells. *PLoS ONE* 6: e17084

Jonsson T, Atwal JK, Steinberg S, Snaedal J, Jonsson PV, Bjornsson S, Stefansson H, Sulem P, Gudbjartsson D, Maloney J, Hoyte K, Gustafson A, Liu Y, Lu Y, Bhangale T, Graham RR, Huttenlocher J, Bjornsdottir G, Andreassen OA, Jönsson EG *et al* (2012) A mutation in APP protects against Alzheimers disease and age-related cognitive decline. *Nature* 488: 96–99

Kim C, Wong J, Wen J, Wang S, Wang C, Spiering S, Kan NG, Forcales S, Puri PL, Leone TC, Marine JE, Calkins H, Kelly DP, Judge DP, Chen HS (2013) Studying arrhythmogenic right ventricular dysplasia with patient-specific iPSCs. *Nature* 494: 105–110

Kim K, Doi A, Wen B, Ng K, Zhao R, Cahan P, Kim J, Aryee MJ, Ji H, Ehrlich LI, Yabuuchi A, Takeuchi A, Cunniff KC, Hongguang H, McKinney-Freeman S, Naveiras O, Yoon TJ, Irizarry RA, Jung N, Seita J *et al* (2010) Epigenetic memory in induced pluripotent stem cells. *Nature* 467: 285–290

Kitaoka S, Kondoh H, Inoue H (2011) Induced pluripotent stem cell technology for the study of neurodegenerative diseases. In *Induced Stem Cells*, Sullivan PJ, Mortensen EK (eds), pp 129–142. New York: Nova Science Publishers Inc

Kobayashi T, Yamaguchi T, Hamanaka S, Kato-Itoh M, Yamazaki Y, Ibata M, Sato H, Lee YS, Usui J, Knisely AS, Hirabayashi M, Nakauchi H (2010) Generation of rat pancreas in mouse by interspecific blastocyst injection of pluripotent stem cells. *Cell* 142: 787–799

Kondo T, Asai M, Tsukita K, Kutoku Y, Ohsawa Y, Sunada Y, Imamura K, Egawa N, Yahata N, Okita K, Takahashi K, Asaka I, Aoi T, Watanabe A, Watanabe K, Kadoya C, Nakano R, Watanabe D, Maruyama K, Hori O *et al* (2013) Modeling Alzheimers disease with iPSCs reveals stress phenotypes associated with intracellular Aβ and differential drug responsiveness. *Cell Stem Cell* 12: 487–496

Lahti AL, Kujala VJ, Chapman H, Koivisto AP, Pekkanen-Mattila M, Kerkelä E, Hyttinen J, Kontula K, Swan H, Conklin BR, Yamanaka S, Silvennoinen O, Aalto-Setälä K (2012) Model for long QT syndrome type 2 using human iPS cells demonstrates arrhythmogenic characteristics in cell culture. *Dis Model Mech* 5: 220–230

Marchetto MC, Gage FH (2012) Modeling brain disease in a dish: really? *Cell Stem Cell* 10: 642–645

Medine CN, Lucendo-Villarin B, Storck C, Wang F, Szkolnicka D, Khan F, Pernagallo S, Black JR, Marriage HM, Ross JA, Bradley M, Iredale JP, Flint O, Hay DC (2013) Developing high-fidelity hepatotoxicity models from pluripotent stem cells. *Stem Cells Transl Med* 2: 505–509

Mekhoubad S, Bock C, de Boer AS, Kiskinis E, Meissner A, Eggan K (2012) Erosion of dosage compensation impacts human iPSC disease modeling. *Cell Stem Cell* 10: 595–609

Merkle FT, Eggan K (2013) Modeling human disease with pluripotent stem cells: from genome association to function. *Cell Stem Cell* 12: 656–668

Mirnezami R, Nicholson J, Darzi A (2012) Preparing for precision medicine. *N Engl J Med* 366: 489–491

Morizane A, Doi D, Kikuchi T, Okita K, Hotta A, Kawasaki T, Hayashi T, Onoe H, Shiina T, Yamanaka S, Takahashi J (2013) Direct comparison of autologous and allogeneic transplantation of iPSC-derived neural cells in the brain of a nonhuman primate. *Stem Cell Reports* 1: 283–292

Nakajima F, Tokunaga K, Nakatsuji N (2007) Human leukocyte antigen matching estimations in a hypothetical bank of human embryonic stem cell lines in the Japanese population for use in cell transplantation therapy. *Stem Cells* 25: 983–985

Nakaki F, Hayashi K, Ohta H, Kurimoto K, Yabuta Y, Saitou M (2013) Induction of mouse germ-cell fate by transcription factors in vitro. *Nature* 501: 222–226

Nakamura M, Okano H (2013) Cell transplantation therapies for spinal cord injury focusing on induced pluripotent stem cells. *Cell Res* 23: 70–80

Nishimura T, Kaneko S, Kawana-Tachikawa A, Tajima Y, Goto H, Zhu D, Nakayama-Hosoya K, Iriguchi S, Uemura Y, Shimizu T, Takayama N, Yamada D, Nishimura K, Ohtaka M, Watanabe N, Takahashi S, Iwamoto A, Koseki H, Nakanishi M, Eto K *et al* (2013) Generation of rejuvenated antigen-specific T cells by reprogramming to pluripotency and redifferentiation. *Cell Stem Cell* 12: 114–126

Okita K, Matsumura Y, Sato Y, Okada A, Morizane A, Okamoto S, Hong H, Nakagawa M, Tanabe K, Tezuka K, Shibata T, Kunisada T, Takahashi M, Takahashi J, Saji H, Yamanaka S (2011) A more efficient method to generate integration-free human iPS cells. *Nat Methods* 8: 409–412

Park IH, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, Lensch MW, Cowan C, Hochedlinger K, Daley GQ (2008) Disease-specific induced pluripotent stem cells. *Cell* 134: 877–886

Qian L, Huang Y, Spencer CI, Foley A, Vedantham V, Liu L, Conway SJ, Fu JD, Srivastava D (2012) In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. *Nature* 485: 593–598

Qiang L, Fujita R, Abeliovich A (2013) Remodeling neurodegeneration: somatic cell reprogramming-based models of adult neurological disorders. *Neuron* 78: 957–969

Qiang L, Fujita R, Yamashita T, Angulo S, Rhinn H, Rhee D, Doege C, Chau L, Aubry L, Vanti WB, Moreno H, Abeliovich A (2011) Directed conversion of Alzheimers disease patient skin fibroblasts into functional neurons. *Cell* 146: 359–371

Rhinn H, Fujita R, Qiang L, Cheng R, Lee JH, Abeliovich A (2013) Integrative genomics identifies APOE ε4 effectors in Alzheimers disease. *Nature* 500: 45–50

Sandoe J, Eggan K (2013) Opportunities and challenges of pluripotent stem cell neurodegenerative disease models. *Nat Neurosci* 16: 780–789

Sasai Y (2013) Next-generation regenerative medicine: organogenesis from stem cells in 3D culture. *Cell Stem Cell* 12: 520–530

Scott CW, Peters MF, Dragan YP (2013) Human induced pluripotent stem cells and their use in drug discovery for toxicity testing. *Toxicol Lett* 219: 49–58

Sekiya S, Suzuki A (2011) Direct conversion of mouse fibroblasts to hepatocyte-like cells by defined factors. *Nature* 475: 390–393

Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, Richards DR, McDonald-Smith GP, Gao H, Hennessy L, Finnerty CC, López CM, Honari S, Moore EE, Minei JP, Cuschieri J, Bankey PE, Johnson JL, Sperry J, Nathens AB *et al* (2013) Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc Natl Acad Sci USA* 110: 3507–3512

Soldner F, Jaenisch R (2012) Medicine. iPSC disease modeling. *Science* 338: 1155–1156

Son EY, Ichida JK, Wainger BJ, Toma JS, Rafuse VF, Woolf CJ, Eggan K (2011) Conversion of mouse and human fibroblasts into functional spinal motor neurons. *Cell Stem Cell* 9: 205–218

Song K, Nam YJ, Luo X, Qi X, Tan W, Huang GN, Acharya A, Smith CL, Tallquist MD, Neilson EG, Hill JA, Bassel-Duby R, Olson EN (2012) Heart

repair by reprogramming non-myocytes with cardiac transcription factors. *Nature* 485: 599 – 604

Szabo E, Rampalli S, Risueño RM, Schnerch A, Mitchell R, Fiebig-Comyn A, Levadoux-Martin M, Bhatia M (2010) Direct conversion of human fibroblasts to multilineage blood progenitors. *Nature* 468: 521 – 526

Takahashi K, Yamanaka S (2013) Induced pluripotent stem cells in medicine and biology. *Development* 140: 2457 – 2461

Tanaka A, Woltjen K, Miyake K, Hotta A, Ikeya M, Yamamoto T, Nishino T, Shoji E, Sehara-Fujisawa A, Manabe Y, Fujii N, Hanaoka K, Era T, Yamashita S, Isobe K, Kimura E, Sakurai H (2013) Efficient and reproducible myogenic differentiation from human iPS cells: prospects for modeling Miyoshi Myopathy in vitro. *PLoS One* 8: e61540

Tobert (2003) Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors *Nat Rev Drug Discov*. 2: 517 – 526

Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Südhof TC, Wernig M (2010) Direct conversion of fibroblasts to functional neurons by defined factors. *Nature* 463: 1035 – 1041

Vizcardo R, Masuda K, Yamada D, Ikawa T, Shimizu K, Fujii S, Koseki H, Kawamoto H (2013) Regeneration of human tumor antigen-specific T cells from iPSCs derived from mature CD8(+) T cells. *Cell Stem Cell* 12: 31 – 36

Wakao H, Yoshikiyo K, Koshimizu U, Furukawa T, Enomoto K, Matsunaga T, Tanaka T, Yasutomi Y, Yamada T, Minakami H, Tanaka J, Oda A, Sasaki T, Wakao R, Lantz O, Udagawa T, Sekiya Y, Higuchi K, Harada N, Nishimura K *et al* (2013) Expansion of functional human mucosal-associated invariant T cells via reprogramming to pluripotency and redifferentiation. *Cell Stem Cell* 12: 546 – 558

Winklhofer KF, Tatzelt J, Haass C (2008) The two faces of protein misfolding: gain- and loss-of-function in neurodegenerative diseases. *EMBO J* 27: 336 – 349

Xu XH, Zhong Z (2013) Disease modeling and drug screening for neurological diseases using human induced pluripotent stem cells. *Acta Pharmacol Sin* 34: 755 – 764

Yamanaka S (2012) Induced pluripotent stem cells: past, present, and future. *Cell Stem Cell* 10: 678 – 684

Yu DX, Marchetto MC, Gage FH (2013) Therapeutic translation of iPSCs for treating neurological disease. *Cell Stem Cell* 12: 678 – 688

Zerhouni EA (2005) US biomedical research: basic, translational, and clinical sciences. *JAMA* 294: 1352 – 1358

Zhang Y, Pak C, Han Y, Ahlenius H, Zhang Z, Chanda S, Marro S, Patzke C, Acuna C, Covy J, Xu W, Yang N, Danko T, Chen L, Wernig M, Südhof TC (2013) Rapid single-step induction of functional neurons from human pluripotent stem cells. *Neuron* 78: 785 – 798

Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA (2008) In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. *Nature* 455: 627 – 632